Published in "Nature Structural & Molecular Biology" 18: 1376–1380, 2011
which should be cited to refer to this work.

Supplementary information

Structural basis for the molecular evolution of SRP-GTPase activation by protein

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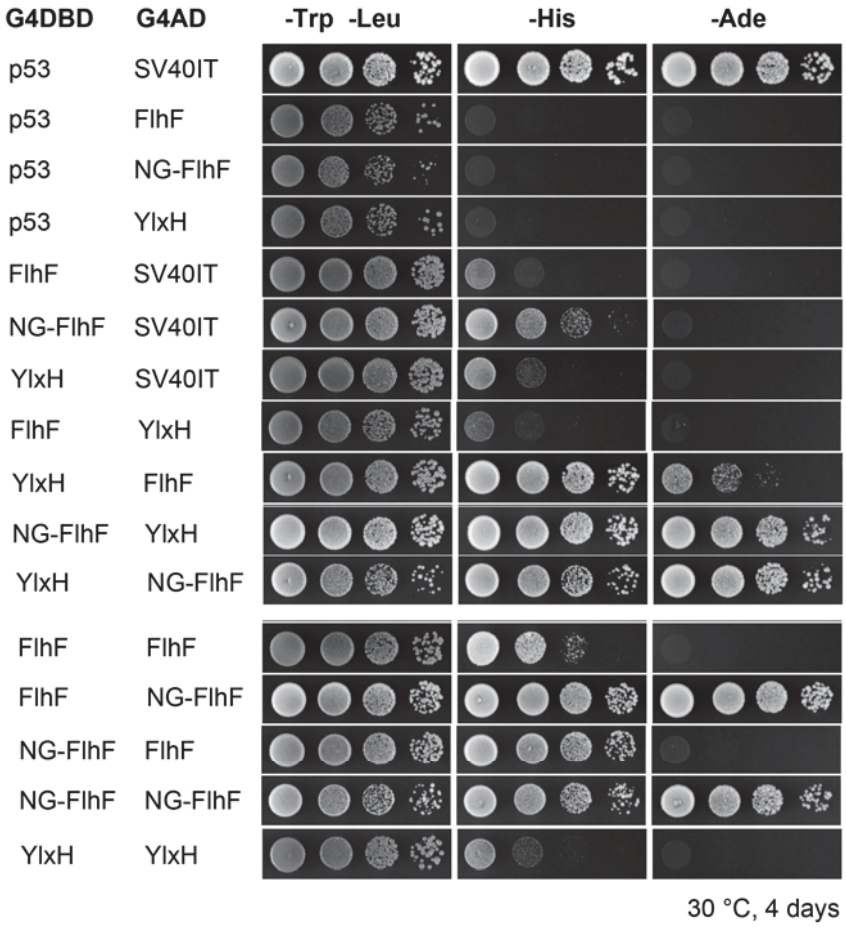
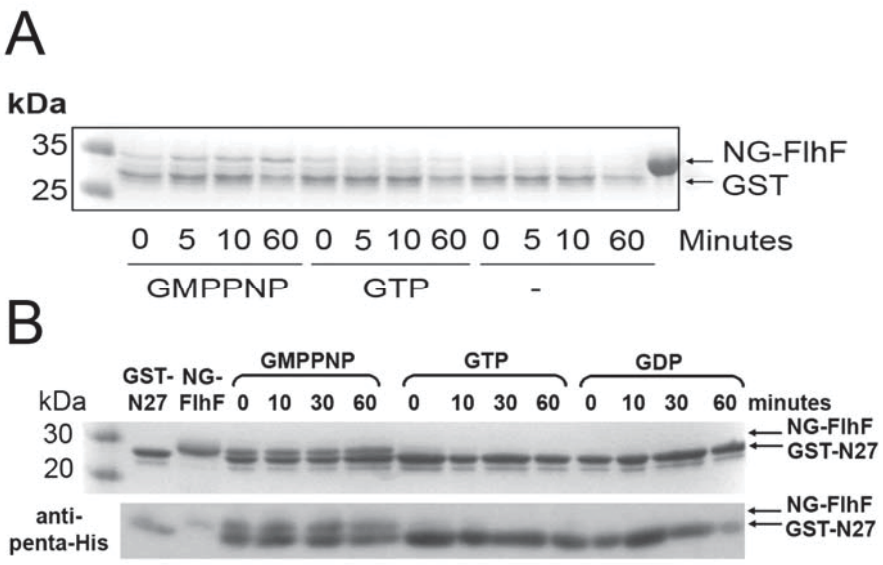
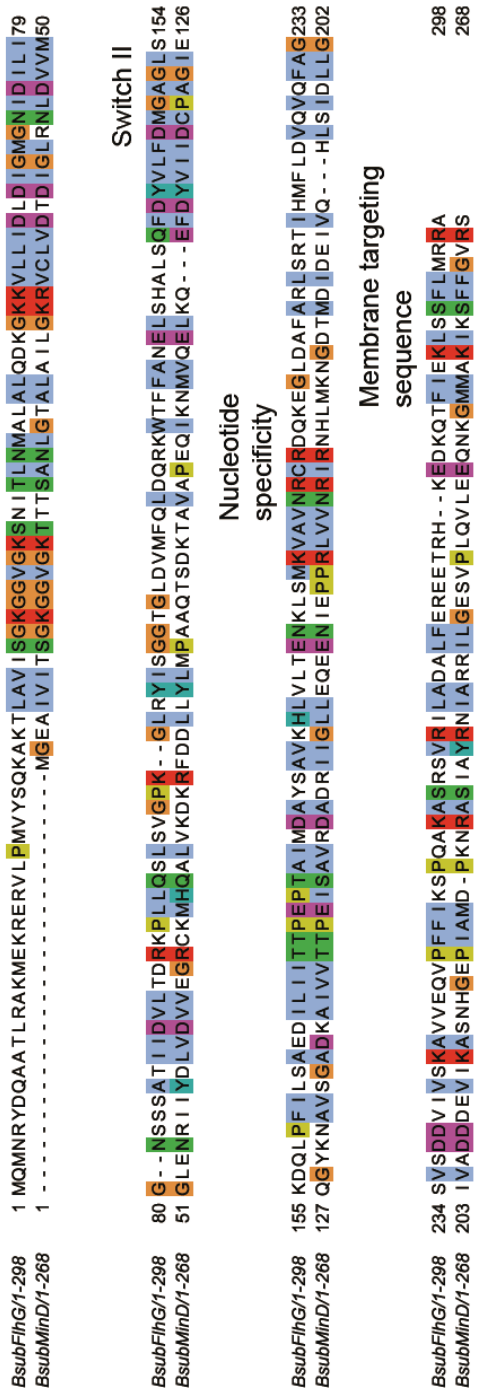


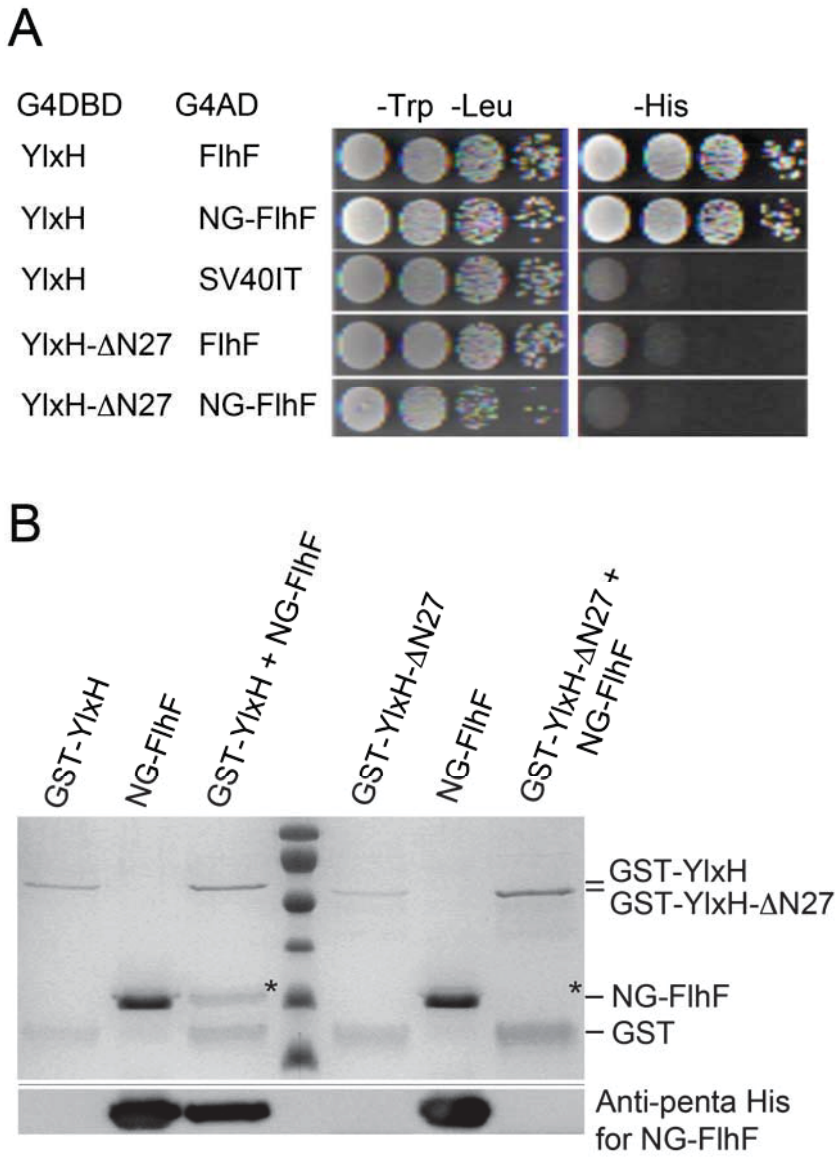
Supplementary Fig. 1. FlhF and NG-FlhF interact with YlxH in the Yeast-two-Hybrid assay. Upper panel: The proteins p53 and SV40IT were used as controls. Full-length FlhF interacts with YlxH, however only in one direction, which suggests a sterical hindrance by the N-terminal B-domain. This effect is not observed for the interaction of NG-FlhF with YlxH. Lower panel: Full-length FlhF shows a weak self-interaction. Combination of FlhF and NG-FlhF shows significant stronger interactions. NG-FlhF shows a strong self-interaction. These observations suggest a regulatory role of the B-domain on FlhF homodimer formation as suggested previously[1] and are reminiscent to the situation of the A-domain in the SRP receptor FtsY[2]. No self-interaction of YlxH was observed.



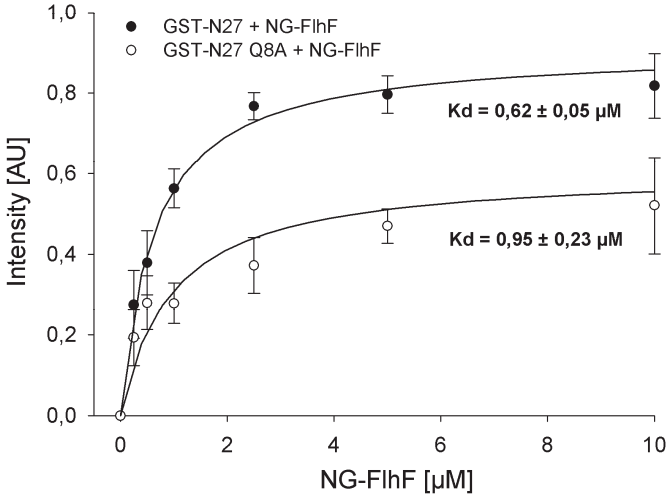
Supplementary Fig. 2. Stability of the FlhF-YlxH interaction in the presence of different nucleotides. **a.** Over a time of 60 minutes the interaction between NG-FlhF and YlxH is only stable in the presence of the non-hydrolysable GTP analogue GMPPNP, but not with GTP or in the absence of nucleotide. **b.** In agreement with a., N27 and NG-FlhF stably interact only in the presence of GMPPNP, but not with GTP or GDP. The nucleotide concentration was 0.25 mM. These experiments suggest that the first 27 amino acid residues of YlxH stimulate the GTPase activity of FlhF.



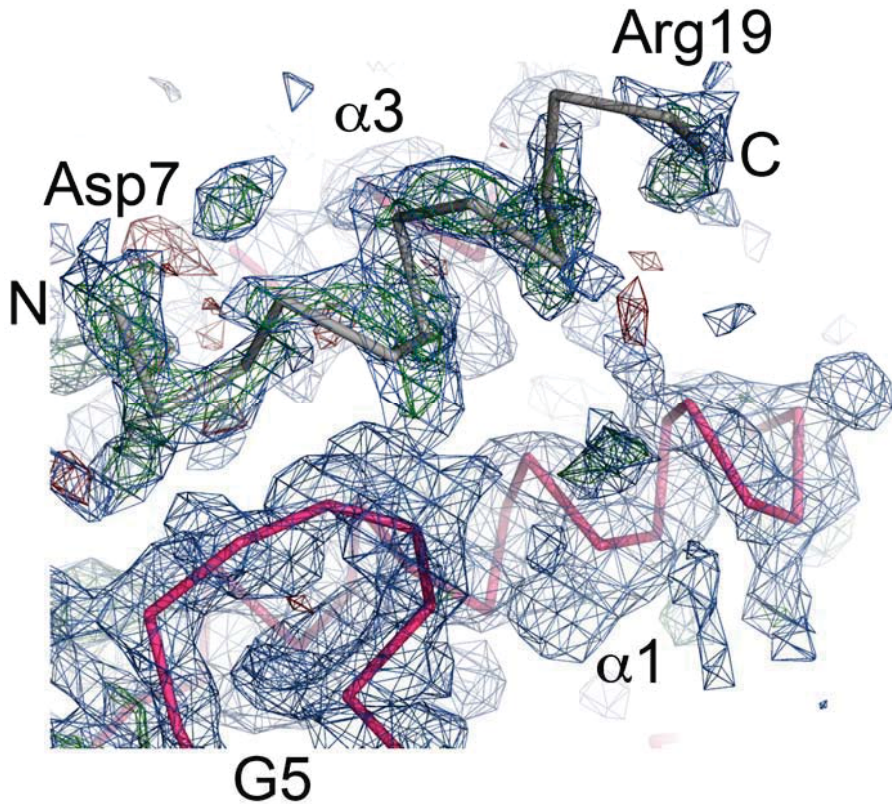
Supplementary Fig. 3. YlxH is a close homologue of MinD. Alignment of YlxH and MinD from *B. subtilis*. MinD and YlxH from *B. subtilis* share 24.6 % sequence identity (73 out of 297 residues) and 23.6 % conservative replacements (70 out 297 residues). Both proteins mainly differ in an N-terminal extension. The characteristic ATPase regions and the membrane targeting sequence are indicated.



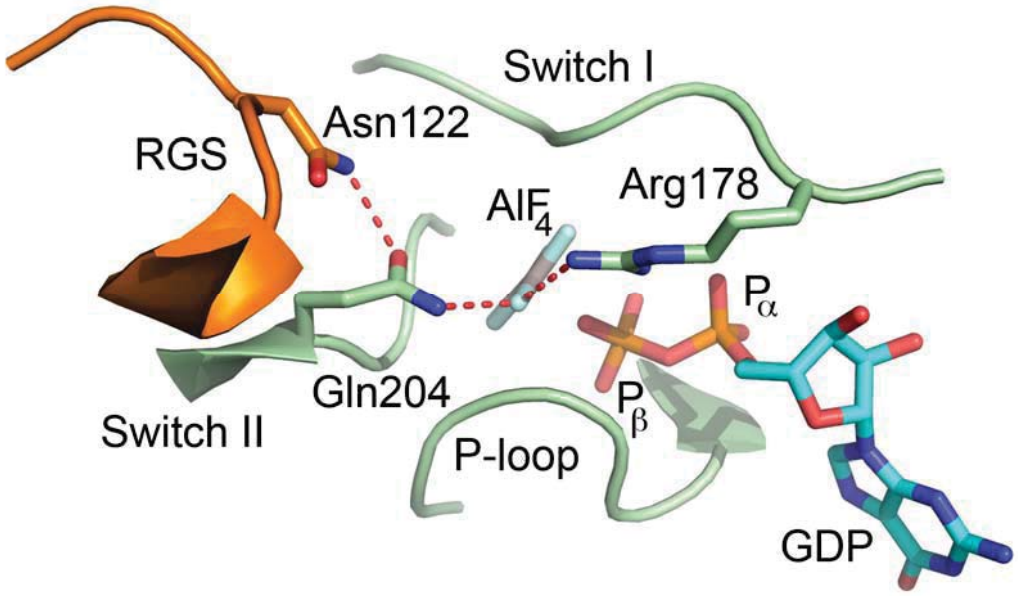
Supplementary Fig. 4. The N-terminus of YlxH is required for its interaction with FlhF. **a.** The Y2H assay shows that the first 27 residues at the N-terminus of YlxH are required for its interaction with FlhF. **b.** The *in vitro* pull-down assay supports these results. A GST-YlxH-DN27 variant is unable to interact with NG-FlhF. All experiments were performed in the presence of the non-hydrolyzable GTP analogue GMPPNP. Asterisks indicate the presence or absence of NG-FlhF, which was confirmed by western blotting against the hexa-histidine tag present in the protein (lower panel).



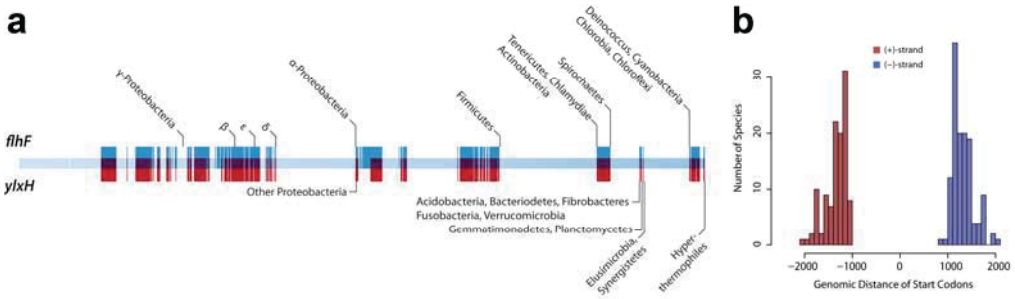
Supplementary Fig. 5. Binding affinities of NG-FlhF to GST-YlxH-N27 and the GST-YlxH-N27-Q8A variant as determined from a GST-pull-down assay. All experiments were performed in triplicates.



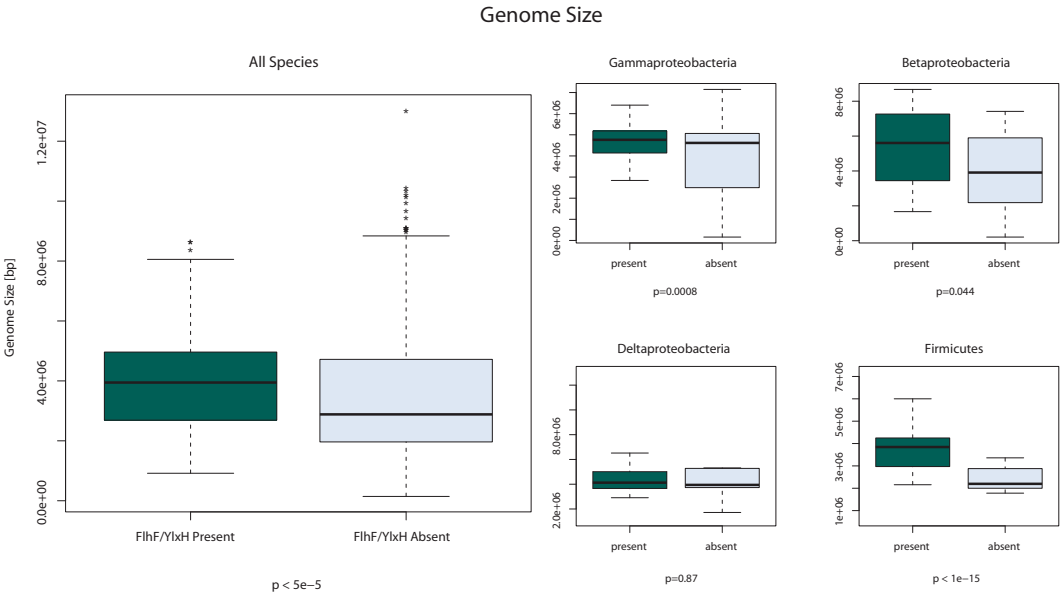
Supplementary Fig. 6. Structure of the enhancer helix (gray) of YlxH bound to FlhF (red) with the contour of a 2Fobs – Fcalc unbiased omit map calculated for the enhancer helix. The difference density Fobs – Fcalc electron density contoured at 3σ, shown as a green mesh. The 2Fobs – Fcalc electron density for the entire complex is contoured at 1.3σ and displayed as blue mesh.



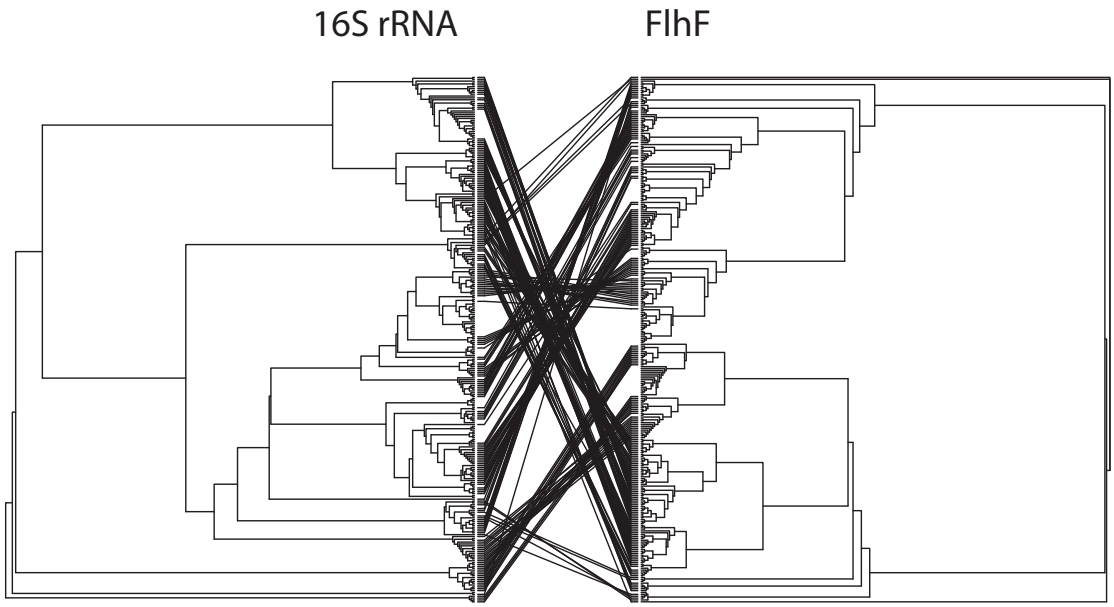
Supplementary Fig. 7. Activation of Gα by RGS. The Gα protein contains the catalytic set of residues, which are required for stabilization of the transition state, e.g. Arg178 and Gln204. The transition state is mimicked by GDP/aluminium tetrafluoride. The RGS does not contribute residues directly involved in catalysis, but orchestrates the stabilization of the transition state by locking the switch regions I and II of Gα into the catalytic position. The conserved Asn122 from RGS stabilizes the side chain of Gln204 to accelerate GTP hydrolysis. The pdb code is: 2ODE[3].



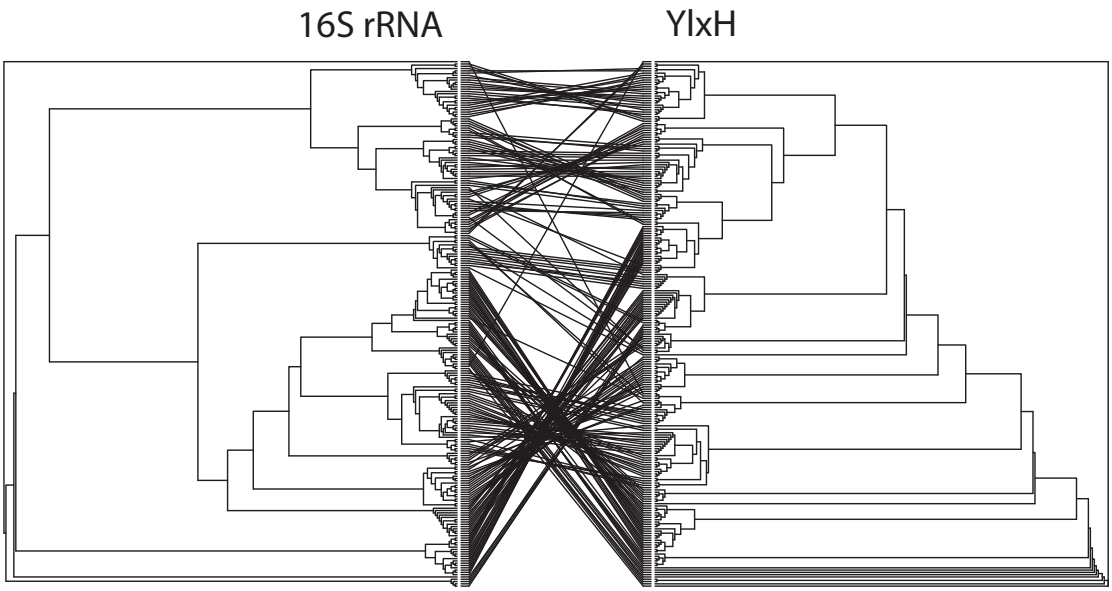
Supplementary Fig. 8. An ancient system for the determination of the future flagellum site. **a.** Phylogenetic pattern of FlhF (blue, up) and YlxH (red, down) mapped on 1111 bacterial species which are in the order of taxonomy as provided by KEGG[4]. FlhF is present in 327 species, YlxH occurs in 288 species, all of which carry FlhF. **b.** *FlhF* and *ylxH* are always direct genomic neighbours. The distance of 1000 – 2000 nucleotides corresponds to the length of *flhF*, which is consistently upstream of *ylxH* in all species carrying both genes.



Supplemental Fig. 9. Genome size of organisms with and without FlhF/YlxH. Left: Organisms harboring the FlhF/YlxH system have significantly larger genomes. Right: Differences in genome size also hold within many clades that have a sufficient number of species with and without the FlhF/YlxH system.



Supplemental Fig. 10: Phylogenetic trees generated from 16S rRNA and FlhF protein sequences. Distance scores were inferred from ClustalW alignment, topology was created by using neighbor joining. Leaves belonging to the same species were linked in order to facilitate the comparison of clustering. The parallel structure of the trees (as indicated by most links connecting one clade of each tree) suggests that FlhF has evolved largely in parallel to the 16S rRNA sequence, rather than having developed independently or through horizontal gene transfer.



Supplemental Fig. 11. Phylogenetic tree generated from 16S rRNA and YlxH protein sequences. Distance scores were inferred from ClustalW alignment, topology was created by using neighbor joining. Leaves belonging to the same species were linked in order to facilitate the comparison of clustering. The parallel structure of the trees (as indicated by most links connecting one clade of each tree) suggests that YlxH has evolved largely in parallel to the 16S rRNA sequence, rather than having developed independently or through horizontal gene transfer.

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